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# Macrophage subtype patterns in protracted asphyxiation

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**Abstract** The question was examined whether protracted asphyxiation is associated with a distinct macrophage subtype pattern in lung tissue. Immunohistochemical preparations of lung specimens were tested with the antibodies MRP8, MRP14, 27E10 and 25F9 in cases of protracted asphyxiation ($n$ = 8) and in "control" groups (hanging: $n$ = 6; peracute deaths: $n$ = 9). MRP8 and MRP14 interstitial cell counts showed a doubling in protracted asphyxiation. Furthermore, clear increases of 27E10 and 25F9 cells (intravascular and interstitial) were found in protracted asphyxiation, both in the intravascular and interstitial compartments. At present the results look promising as to an additional diagnostic criterion for the differential diagnosis between acute and protracted asphyxiation.

**Key words** Macrophages · Subtypes · Asphyxiation

## Introduction

Expressed proliferation of alveolar/lung macrophages with transition to polynuclear giant cells have repeatedly been attributed to protracted asphyxiation (Janssen 1963; Janssen und Bärtschi 1964; Janssen 1984; Reh 1965). More recently, these findings have been questioned (Betz et al. 1993, 1994) because after counting the number of polynuclear cells, significant differences between protracted asphyxiation and controls were not confirmed. Acute obstructive asphyxiation can also be associated with a highly typical pattern of findings named "hemorrhagic-dysoric syndrome" (Brinkmann 1978; Brinkmann and Püschel 1981). This designation stands for the combination of alveolar interstitial, septal, periductal and intra-alveolar hemorrhagic edema in combination with micro-hemorrhages and intra-vascular cell aggregation (myeloic cells, mononuclear cells and platelets either in combination or in isolation).

Various defined macrophage subtypes have been elaborated and consequently ascribed to early and late stages of inflammation (Zwadlo et al. 1985a, b, 1986; Sorg 1991; Mahnke et al. 1995). Defined macrophage subtype patterns can therefore reflect a given stage of the inflammatory process. We have therefore examined the question whether protracted asphyxiation is associated with a distinct macrophage subtype pattern.

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## Materials and methods

For this investigation, three peripheral lung specimens from three different lobes (middle lobe, right or left superior and inferior lobes) were fixed in buffered formalin and embedded in paraffin. Autopsies had been performed up to two days post mortem. After preliminary conventional histology cases with advanced autolysis or with pre-existing inflammatory lung disease were excluded giving the following groups:

1. Protracted asphyxiation including eight cases with estimated survival times between 15 min and 1 h (individual data see Table 1).

2. Hanging including six cases with estimated survival times of approximately 5 min (Table 1).

3. Short agony controls including nine cases (estimated survival less than 2 min Table 1).

Sections from each paraffin block were stained separately for four macrophage markers.

### Cell counts

From each section 12 visual fields were randomly selected and the positive cells counted at a magnification of 200-fold. For cell differentiation a 400-fold magnification was used. Only peripheral areas were considered and two compartments were evaluated separately, i.e. the intravascular and the interstitial compartment. Intra-

**Table 1** Individual data for the groups with a. protracted asphyxiation b. hanging and c. peracute deaths containing information on sex, age and mechanism of death

**Table 1a** Cases with protracted asphyxiation

| Case | Sex | Age | Mechanism |
|---|---|---|---|
| 1 | F | 26 years | Death in plastic sack |
| 2 | M | 7 months | Wrapped in a bed tick |
| 3 | M | 2,5 years | Squashed in a folding bed |
| 4 | M | 28 years | Thorax compression, unusual belt construction |
| 5 | F | 8 years | Accidental death in a wooden chest |
| 6 | F | 10 years | Accidental death in a wooden chest |
| 7 | F | 2 years | Squeezing of head and neck between bars of a iron gate |
| 8 | M | 65 years | Suffocated under plastic cover |

**Table 1b** Hanging cases

| Case | Sex | Age |
|---|---|---|
| 1 | M | 25 years |
| 2 | M | 31 years |
| 3 | M | 32 years |
| 4 | M | 25 years |
| 5 | M | 25 years |
| 6 | M | 52 years |

**Table 1c** Peracute death cases (caused by severe destruction of the brain or by polytrauma)

| Case | Sex | Age | Mechanism |
|---|---|---|---|
| 1 | M | 24 years | Run over (railway) |
| 2 | M | 25 years | Shot |
| 3 | F | 8 years | Traffic accident |
| 4 | M | 51 years | Shot |
| 5 | M | 54 years | Shot |
| 6 | F | 29 years | Traffic accident |
| 7 | M | 59 years | Shot |
| 8 | M | 60 years | Traffic accident |
| 9 | M | 30 years | Traffic accident |

alveolar macrophages were not considered. Only macrophages were counted and granulocytes were disregarded. The arithmetic mean values for the cases, (cells per field of view, after cell counting in 3 × 12 fields) and the mean values of the case groups with the standard deviation were calculated.

## Immunohistochemistry

Paraffin sections 3–4 mm thick were used. The markers MRP 8, MRP 14, 27E10 and 25F9 (Dianova) were visualised using the avidin-biotin-complex method (ABC-method; Nishi et al. 1988). Enzyme pretreatment with proteinase K was advantageous with 25F9 and 27E10 and therefore applied for these markers.

The primary antibodies were used at a dilution of 1:50 (MRP8 and MRP14) or 1:25 (25F9 and 27E10). As described in the Dianova protocol, the antibodies MRP14, MRP8 and 25F9 are stable to formaldehyde fixation and paraffin embedding, but there are conflicting reports with respect to the application of 27E10 with paraffin-embedded and formaldehyde-fixed tissue sections.

## Characterization of macrophage markers

MRP 8 and MRP 14 (MIF related protein) are calcium binding proteins expressed in the late and early activation stages of myelomonocytic differentiation. They are expressed intracellularly in monocytes and granulocytes but not in lymphocytes and mature tissue macrophages. In stimulated monocyte cultures, MRP 14 cells become positive after a few hours. After experimental stimulation in mice, cells with MRP 14 expression increased after 1 h (Zwadlo et al. 1985a; Odnik et al. 1987; Sunderkötter et al. 1990; Roth and Sorg 1992; Hessian et al. 1993).

27E10 is the heterodimer of MRP 8 and MRP 14. Contrary to the intracellular localisation of the monomers, the 27E10-epitope is expressed on the cell surface. The antigen is expressed in subpopulations of macrophages, monocytes and granulocytes. 27E10-positive macrophages also appear in the early stages of inflammation. In stimulated monocyte cultures, the expression of 27E10

**Table 2** Macrophage counts (average cell number per field of view) after immunohistochemical preparation with the antibodies MRP8, MRP14, 27E10 and 25F9 in cases of protracted asphyxiation, peracute death and hanging. The macrophage counts are given separately for the intravascular an interstitial compartments. ( ) = standard deviation

| | 27E10 | MRP8 | MRP14 | 25F9 |
|---|---|---|---|---|
| Protr. asphyx. $n = 8$ | | | | |
| intravasc. | 3.3 (3.9) | 7.8 (5.1) | 6.8 (4.4) | 3.6 (4.2) |
| interst. | 5.1 (5.1) | 19.5 (14.7) | 19.3 (12.3) | 6.4 (5.1) |
| Perac. death $n = 9$ | | | | |
| intravasc. | 0.3 (0.9) | 8.7 (4.6) | 4.0 (1.6) | 0.0 (0.2) |
| interst. | 0.3 (0.7) | 13.5 (4.5) | 7.6 (5.4) | 0.0 (0.1) |
| Hanging $n = 6$ | | | | |
| intravasc. | 0.6 (0.9) | 7.6 (2.0) | 3.4 (1.1) | neg. |
| interst. | 0.5 (1.0) | 9.5 (1.8) | 6.6 (2.5) | 0.2 (0.3) |

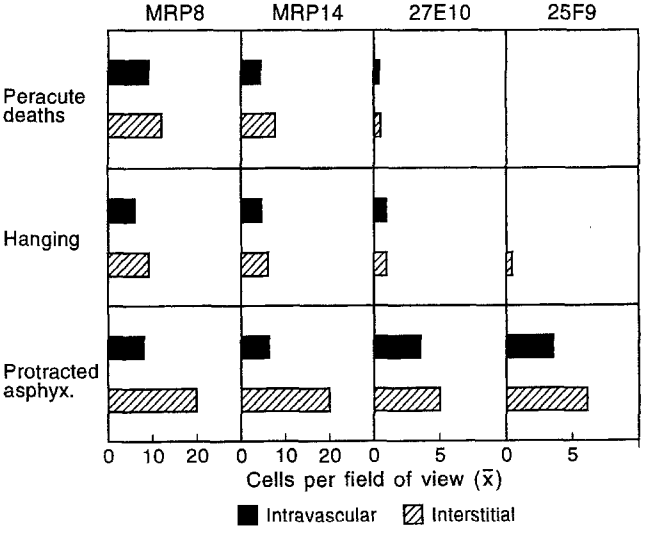


**Fig. 1** Results of macrophage counts as given in Table 2

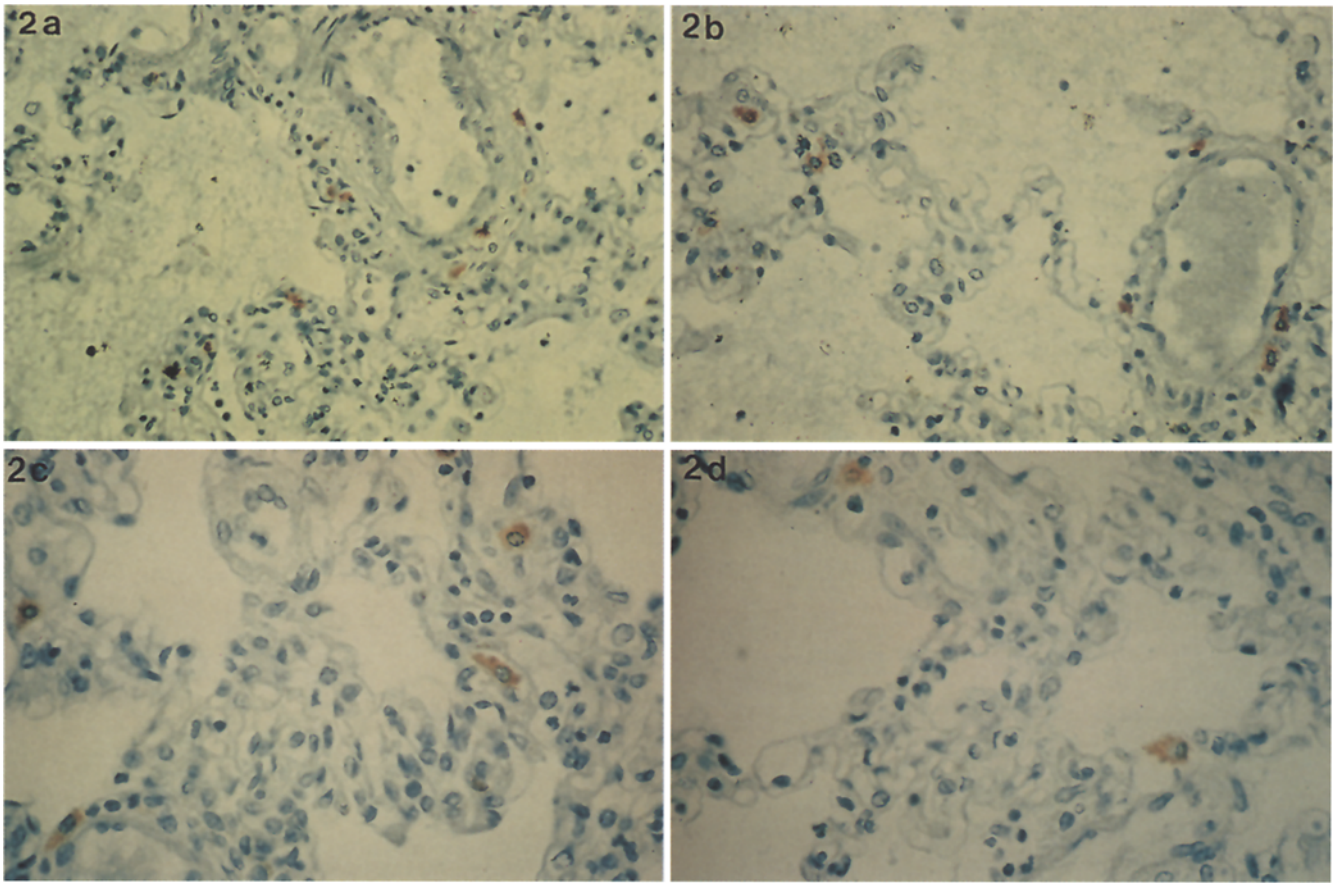


**Fig. 2a–d** Protracted asphyxiation in a wooden chest, lung tissue. **a** girl, 8 years old. 1:200, intravascular and interstitial macrophages, MRP8 positive. **b** girl, 10 years old. 1:200, intravascular and interstitial macrophages, MRP14 positive. **c** girl, 8 years old. 1: 400, intravascular and interstitial macrophages, 27E10 positive. **d** girl, 10 years. 1: 400, intravascular and interstitial makrophages, 25F9 positive

shows a maximum on the 2nd and 3rd day and diminishes thereafter. The epitope is lacking in chronic inflammation and is not expressed in lymphocytes, platelets and mature tissue macrophages. In monocyte cultures, the addition of the $Ca^{2+}$ ionophor A23187 was associated with a rise in 27E10 expression after a few minutes (Bhardwaj et al. 1992).

25F9 is a late stage marker of inflammation expressed in mature macrophages and lacking in young monocytes and also in other blood cells. In stimulated monocyte cultures, the expression increases from the third day on and reaches the maximum after 8–9 days (Zwadlo et al. 1985b).

## Results

With the staining procedure used, granulocytes could easily be distinguished from monocytes. As was to be expected (Sorg 1991) MRP8, MRP14 and 27E10 reacted to a greater or lesser extent with (intravascular) blood monocytes in both "control" groups and in the protracted asphyxiation group. 25F9 showed a weak reaction with monocytes only in the protracted asphyxiation group. Only cells in the small vessels were counted where the maximum sizes did not exceed those of vessels accompanying the terminal bronchioles.

MRP 8 cell counts were approximately in the same range in all three groups (Table 2, Fig. 1) with the exception of MRP $8^{interst.}$ in protracted asphyxiation where we observed on average a doubling MRP 14 cell counts were similar to MRP 8 (Table 2, Fig. 1); i.e. a doubling of MRP $14^{interst.}$ in protracted asphyxiation.

27E10 counts were extremely low in both „control" groups and increased to a severalfold in protracted asphyxiation and in both compartments (Table 2, Fig. 1).

## Discussion

From the literature it is difficult to predict the results associated with our investigation. We did not expect the level of differences between fast and protracted deaths, either for the so-called early stage markers MRP 8, MRP 14, 27E10 or for the so-called late stage marker 25F9. The reaction times reported in the literature were significantly higher, but it must be emphasized that these were mainly derived from cultured cells or other tissues in vivo (e.g. skin). Only the reaction times reported by Sunderkötter et al. (1991) after experimental cauterization of mice corneae and by Bhardwaj et al. (1992) with the addition of Ca ionophors A23187 resulted in comparable time levels at least for selected early stage markers. Comparisons of our data with results obtained from standard experiments with cultured cells and selected tissues led us to consider the involvement of additional trigger mechanisms acceler-

ating the activation process of macrophages. These can either be mechanisms of systemic humoral response or of local responses. Berg (1963) reported a multifold increase of serum catecholamines in acute asphyxiation but other messenger molecules must also be considered e.g. the local release of peptides and kinines and other mediators from endothelial cells after hypoxic damage.

In protracted asphyxiation an initial slight increase of the markers MRP 8 and MRP 14 occurs in the alveolar, septal and periductal interstitium. The complex early stage marker 27E10 shows a several-fold increase in both compartments and the late stage marker 25F9 is associated with a multifold increase of the cell counts.

The explanation of the sequence is possibly simple: MRP 8 and MRP 14 cells seem to be present in considerable numbers in both compartments in short agony "controls" and there occurs a first "reaction" only in the pulmonary interstitium in cases of protracted asphyxiation. Since the other two markers are either absent or associated with very low cell counts in both "control" groups the increase can only be explained by activation and release from bone marrow because they are increased in both compartments.

The intra-alveolar macrophages were difficult to evaluate statistically because there was a high proportion of negative cells and the well known wide variation of the overall cell counts which is due to several reasons (e.g. smoker cells etc). Therefore we would not yet discuss the controversy mentioned in the introduction with proliferation of intra-alveolar cells (Betz et al. 1993, 1994) but protracted asphyxiation is associated with enhanced activation of interstitial and intravascular macrophages.

The diagnostic value of this investigation is at present difficult to predict. While the case-associated individual values of 25F9 did not show an overlap between the control groups and protracted asphyxiation; there were individual overlaps between MRP 8, MRP 14 and 27E10. Therefore more control and protracted cases and also other defined asphyxiation groups (throttling, manual strangulation) should be investigated before coming to any conclusions. At present the results look promising for an additional diagnostic criterion for a very specific differential diagnosis.

## References


Berg St (1963) Physiologisch-chemische Befunde im Leichenblut als Ausdruck des Todesgeschehens. Dtsch Z Gerichtl Med 54: 136–142

Betz P, Beier G, Eisenmenger W (1994) Pulmonary giant cells and traumatic asphyxia. Int J Legal Med 106:258–261

Betz P, Nerlich A, Penning R, Eisenmenger W (1993) Pulmonary giant cells and their significance for the diagnosis of asphyxiation. Int J Legal Med 106:156–159

Bhardwaj R, Zotz C, Zwadlo-Klarwasser G, Roth J, Goebeler M, Mahnke K, Falk M, Meinardus-Hager G, Sorg C (1992) The calcium binding proteins MRP8 and MRP14 form a membrane-associated heterodimer in a subset of monocytes/macrophages present in acute but absent in chronic inflammatory lesions. Eur J Immunol 22:1891–1897

Brinkmann B (1978) Vitale Reaktionen in der Lungenstrombahn bei Tod durch Strangulation. Z Rechtsmedizin 81:133–146

Brinkmann B, Püschel K (1981) Die Lunge als Erfolgsorgan der Strangulationsagonie. Z Rechtsmedizin 86:175–194

Hessian PA, Edgeworth J, Hogg N (1993) MRP 8 and MRP 14 two abundant $Ca^{2+}$-binding proteins of neutrophils and monocytes. J Leukocyte Biol 53:197–204

Janssen W (1963) Riesenzellenbildung bei Erstickung. Dtsch Z Ges Gerichtl Med 54:200–210

Janssen W (1984) Forensic histopathology. Springer, Berlin Heidelberg NewYork

Janssen W, Bärtschi G (1964) Vitale und supravitale Reaktionen der Alveolarzellen nach protrahiertem Sauerstoffmangel. Dtsch Z Ges Gerichtl Med 55:47–66

Mahnke K, Bhardwaj R, Sorg C (1995) Heterodimers of the calcium-binding proteins MRP8 and MRP14 are expressed on the surface of human moncytes upon adherence to fibronectin and collagen. Relation to TNF-a, IL-6, and superoxide production. J Leukoc Biol 57:63–71

Nishi K, Rand S, Fechner G, Brinkmann B (1988) Immunohistochemical localization of A, B, H, Le a and Le b antigens in human thymus using avidin-biotin complex technique. Acta Crim Jpn 54:217–223

Odnik K, Cerletti N, Brüggen J, Clerc RG, Tacsay L, Zwadlo G, Gerhards G, Schlegel R, Sorg C (1987) Two calcium-binding proteins in infiltrate macrophages of rheumatoid arthritis. Nature 330:80–82

Reh H (1965) Die Verfettung der Alveolarepithelien beim plötzlichen Tod. Beitr Gerichtl Med 22:236–242

Roth J, Sorg C (1992) Die kalziumbindenden Proteine MRP8 und MRP14: Zwei neue Marker zur Monozytendifferenzierung in Entzündungen. Atemw Lungenkrkh 18:349–352

Sorg C (1991) Macrophages in acute in chronic inflammation. Chest Suppl 100:173–175

Sunderkötter C, Beil W, Roth J, Sorg C (1991) Cellular events associated with inflammatory angiogenesis in the mouse cornea. Am J Pathol 138:931–939

Zwadlo G, Brüggen J, Gerhards G, Schlegel R, Sorg C (1985a) Two calcium-binding proteins associated with specific stages of myeloid cell differentiation are expressed by subsets of macrophages in inflammatory tissues. Clin Exp Immunol 72: 510–515

Zwadlo G, Bröcker E-B, v Bassewitz D-B, Feige U, Sorg C (1985b) A monoclonal antibody to a differentiation antigen present on mature human macrophages and absent from monocytes. J Immunol 134:1487–1492

Zwadlo G, Schlegel R, Sorg C (1986) A monoclonal antibody to a subset of human monocytes found only in the peripheral blood and inflamatory tissues. J Immunol 137:512–518